Pergamon

Tetrahedron 56 (2000) 9937–9944

TETRAHEDRON

# Structure of Saframycin R

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Received 22 September 2000; accepted 16 October 2000

**Abstract**—The structure of saframycin R was determined to be **1** (form **I**) by the two-dimensional $^{1}$H detected heteronuclear correlation experiments (HMQC and HMBC) of its acylated compounds **4a** and **4b**. 

Saframycin is a class of antibiotics with activity against gram-positive bacteria and also against several kinds of tumor cells.[1] A similar group of metabolites that includes renieramycins and ecetinascidins was isolated from marine sources.[2,3] Although saframycin A (**2**) is one of the most biologically active components of the groups, it is highly

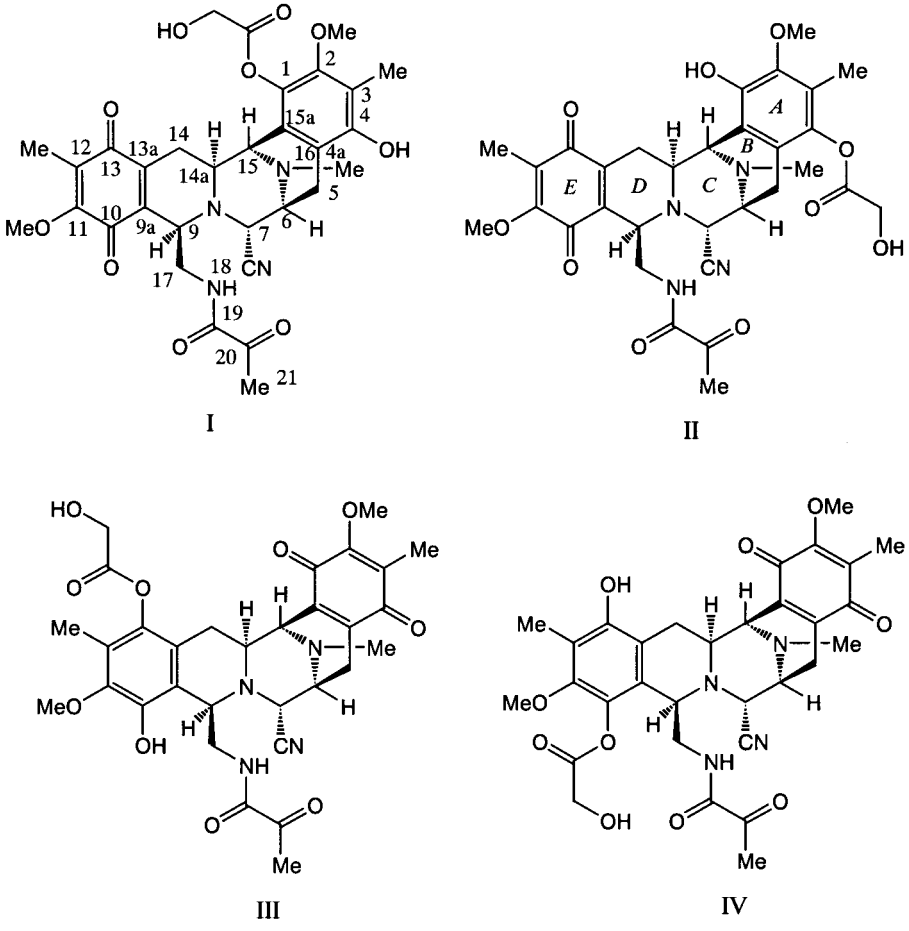


**Figure 1.**

*Keywords*: structure; saframycin R; saframycin A; transformation.

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0040–4020/00/$ - see front matter 
PII: S0040-4020(00)00972-8

**Table 1.** NMR Data for saframycin R (**1**) (all data were recorded in $CDCl_3$)

| Atom no. | $^{13}C$ NMR $\delta_{mult}$ | $^{1}H$ NMR (mult. Integral, *J* (Hz)) | Correlations from C no. |
|---|---|---|---|
| 1 | 135.7 s | | 15-H |
| 2 | 148.3 s | | 2-OMe, 3-Me |
| 3 | 118.1 s | | 3-Me |
| 4 | 149.1 s | | 3-Me, 5-Hβ, 5-Hα |
| 4a | 116.8 s | | 5-Hβ, 5-Hα, 6-H<br>15-H |
| 15a | 121.9 s | | 5-Hβ, 5-Hα, 14a-H<br>15-H |
| 5 | 20.8 t | 2.43 (d, 1H, 17.7)<br>2.93 (dd, 1H, 17.7, 8.1) | 6-H, 7-H |
| 6 | 54.5 d | 3.48 (m, 1H) | 5-Hβ, 5-Hα, 7-H<br>N-Me, 15-H |
| 7 | 58.9 d | 4.07 (d, 1H, 2.4) | 5-Hβ, 5-Hα, 6-H<br>9-H, 14a-H |
| 9 | 56.9 d | 3.93 (ddd, 1H, 2.7, 2.7, 2.0) | 14a-H, 17-$H_2$ |
| 9a | 135.3 s | | 9-H, 17-$H_2$, 14-Hβ<br>14-Hα |
| 10 | 180.8 s | | |
| 11 | 155.9 s | | 11-OMe, 12-Me |
| 12 | 128.4 s | | 12-Me |
| 13 | 185.6 s | | 12-Me, 14-Hα |
| 13a | 141.8 s | | 14-Hβ, 14-Hα, 9-H |
| 14 | 24.3 t | 1.54 (ddd, 1H, 17.7, 11.3, 2.7)<br>2.85 (dd, 1H, 17.7, 2.5) | 15-H |
| 14a | 54.3 d | 3.11 (ddd, 1H, 11.3, 2.7, 2.5) | 7-H, 14-Hα, 15-H |
| 15 | 56.7 d | 3.69 (dd, 1H, 2.7, 0.5) | 6-H, N-Me |
| 17 | 40.4 t | 3.48 (m, 2H) | N–H |
| 19 | 160.5 s | | N–H, 17-$H_2$, COMe |
| 20 | 195.7 s | | COMe |
| 21 | 24.3 q | 2.20 (s, 3H) | |
| 2-OMe | 61.0 q | 3.71 (s, 3H) | |
| 11-OMe | 61.1 q | 4.02 (s, 3H) | |
| 3-Me | 9.2 q | 2.20 (s, 3H) | |
| 12-Me | 8.7 q | 1.93 (s, 3H) | |
| 16-Me | 41.5 q | 2.28 (s, 3H) | 6-H, 15-H |
| CN | 117.0 s | | 7-H |
| CO*C*$H_2$OH | 60.7 t | 4.56 (s, 2H) | |
| *C*OCH$_2$OH | 171.7 s | | C*H*$_2$OH |
| NH | | 6.33 (t, 1H, 6.7) | |
| OH[a] | | 5.28 (br s, 1H) | |

[a] One OH proton was not detected.

toxic, which prevents it from gaining wider acceptance for cancer chemotherapy.[4,5†] Arai and co-workers have investigated minor components of **2** cultures of *Streptomyces lavendulae* No. 314, and discovered saframycin R (**1**), which is active against several experimental tumor cells and the acute toxicity in mice is one tenth that of **2**.[6] The present paper describes the structure of saframycin R determined by two-dimensional $^{1}H$ detected heteronuclear correlation experiments, thus precluding efforts to obtain suitable crystals for an expensive X-ray analysis.

In 1982, Arai and co-workers presented four possible structures (**I**–**IV**) for saframycin R based on its chemical and spectroscopic data (Fig. 1).[6a] The proposed structure was corroborated by the $^{13}C$ NMR spectrum showing 31 distinct resonances, 15 $sp^3$, 15 $sp^2$, and 1 sp carbons, whose multiplicity (6×$CH_3$, 4×$CH_2$, 5×CH, and 16×C) was determined by spin-echo $^{13}C$ effects. Lown et al. further elucidated the structure of saframycin R by comparing the $^{1}H$ NMR spectral data of saframycin A (**1a**).[6b] They ruled out **I** and **II** by comparing the chemical shift differences between the groups of protons adjacent to the E ring (such as 9-H, 14-Hβ, 14-Hα) and those immediately adjacent to ring A (such as 5-Hβ, 5-Hα, 15-H) in saframycins R and A. Saframycin R experiences shifts of 0.20 to 0.10 ppm whereas saframycin A experiences smaller shifts of 0.08–0.00 ppm. Furthermore, the average conformation of the side chain is similar in saframycin R and in saframycin A, which argues against any steric effects due to a large group at C-13 and favors the orientation of form **III**.

For the purposes of discussion of the NMR spectra of saframycin R, the 14 protons (excluding the methyl groups and 2 OH protons) have been reassigned as shown in Table 1. The diagnostic homoallylic coupling (2.7 Hz) between 9-H and 14-Hβ through five bonds was confirmed. In our previous work, this coupling was negligible when the compound did not have quinone functionality at the E ring.[7] Thus, the data allow **III** and **IV** to be ruled out unequivocally. Additional evidence is provided by

† Ecteinascidin 743 is an exceedingly potent antitumor agent obtained from marine ascidian that is currently undergoing phase II clinical trials as a result of its promising efficacy in preclinical antitumor tests. Recently, the Harvard group has found a structural analogue of ecteinascidin 743, phthalascidin, which exhibits antitumor activity essentially indistinguishable from that of the natural product.[5a]

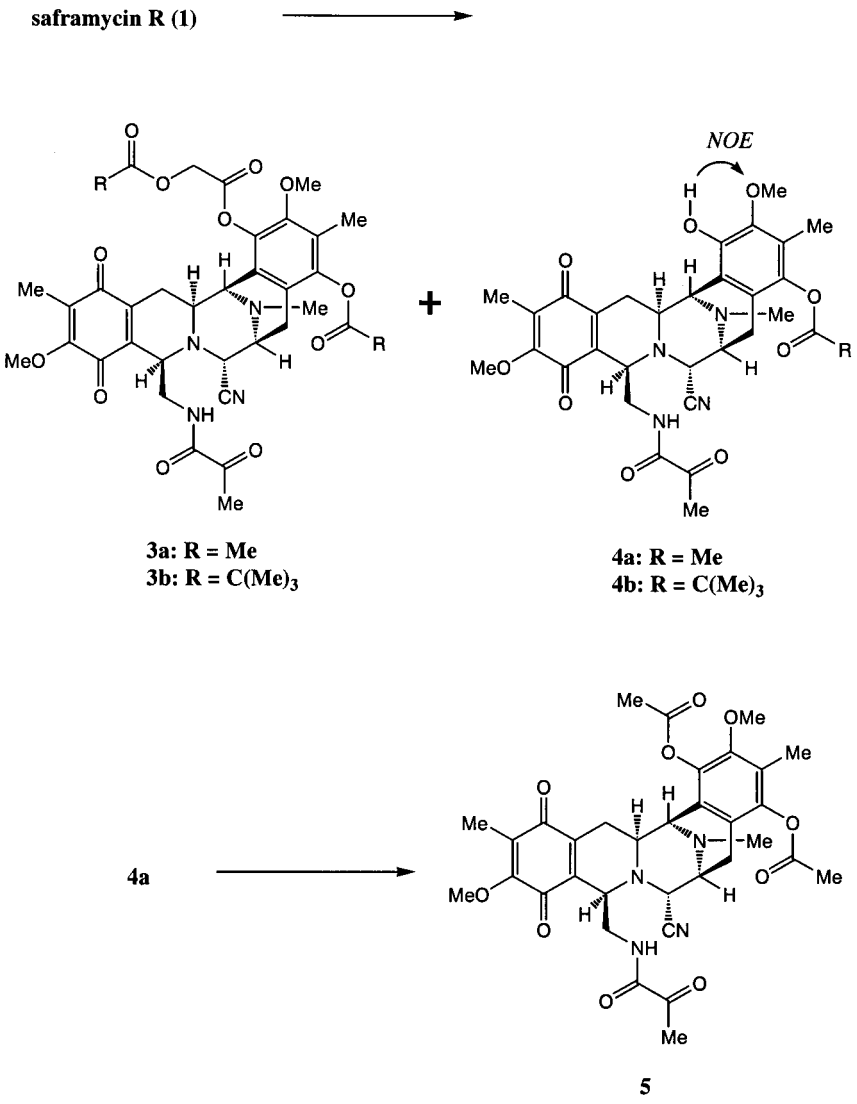



**Chart 1.**

long-range $^{1}H-^{13}C$ connectivity, which was determined through a series of $^{1}H$ detected two-dimensional hetero-nuclear multiple-bond correlation (HMBC) experiments.[8]

The aromatic substituents of the A ring were assigned as follows: The signal at $\delta$ 149.1 was assigned to C-4 based on long-range $^{1}H-^{13}C$ correlations observed between C-4 and the 3-$CH_3$ protons and 5-H$\beta$ and 5-H$\alpha$. A methyl group ($\delta$ 9.2) was located on C-3 ($\delta$ 118.1) based on long-range $^{1}H-^{13}C$ correlations between the 3-$CH_3$ protons and the three carbons C-2, C-3, and C-4. A methoxyl group ($\delta$ 61.0) was attached to C-2 ($\delta$ 148.3) based on a long-range $^{1}H-^{13}C$ correlation between the 2-$OCH_3$ protons and C-2. The signal at $\delta$ 135.7 was assigned to C-1 based on a long-range $^{1}H-^{13}C$ correlation between 15-H and C-1. The signal at $\delta$ 121.9 was assigned to C-15a based on long-range $^{1}H-^{13}C$ correlations between C-15a and the following protons; 5-H$\beta$, 5-H$\alpha$, 15-H, and 14a-H. The signal at $\delta$ 116.8 was assigned to C-4a based on long-range $^{1}H-^{13}C$ correlations between C-4a and the four protons 5-H$\beta$, 5-H$\alpha$, 6-H, and 15-H.

The substituents of ring E were assigned as follows: While two quinone carbonyls appear in the spectrum at $\delta$ 185.6 and $\delta$ 180.8, one quinone carbonyl (C-13) was assigned based on long-range $^{1}H-^{13}C$ correlations between C-13 and 12-$CH_3$ protons and 14-H$\alpha$, and in the other quinone carbonyl (C-10), there were negligible long-range $^{1}H-^{13}C$ correlations. The signal at $\delta$ 135.3 was assigned to C-9a based on long-range $^{1}H-^{13}C$ correlations between C-9a and the following protons; 9-H, 17-$CH_2$, 14-H$\beta$, and 14-H$\alpha$. The signal at $\delta$ 141.8 was assigned to C-13a based on long-range $^{1}H-^{13}C$ correlations between C-13a and the three protons (14-H$\beta$, 14-H$\alpha$, and 9-H).

Thus, there are two possible orientations of the glycolic ester substituents at C-1 or C-4. Unfortunately, there were no confirmatory nuclear Overhauser enhancement (NOE) effects between the phenolic proton and the protons of 2-$OCH_3$ or the phenolic proton and the protons of 3-$CH_3$, because the phenolic proton signal was overlapped with other signals and could not be assigned. We then attempted to obtain derivatives with suitable crystals for X-ray analysis.

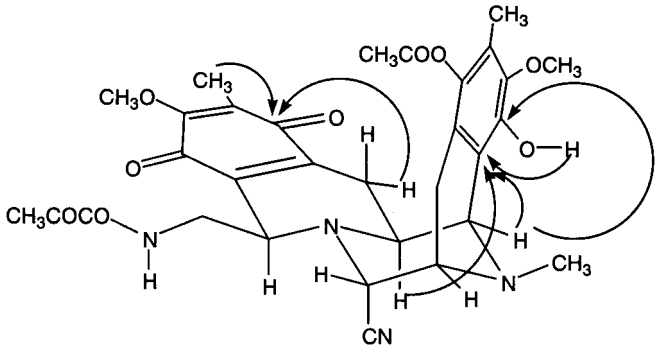


**Figure 2.**

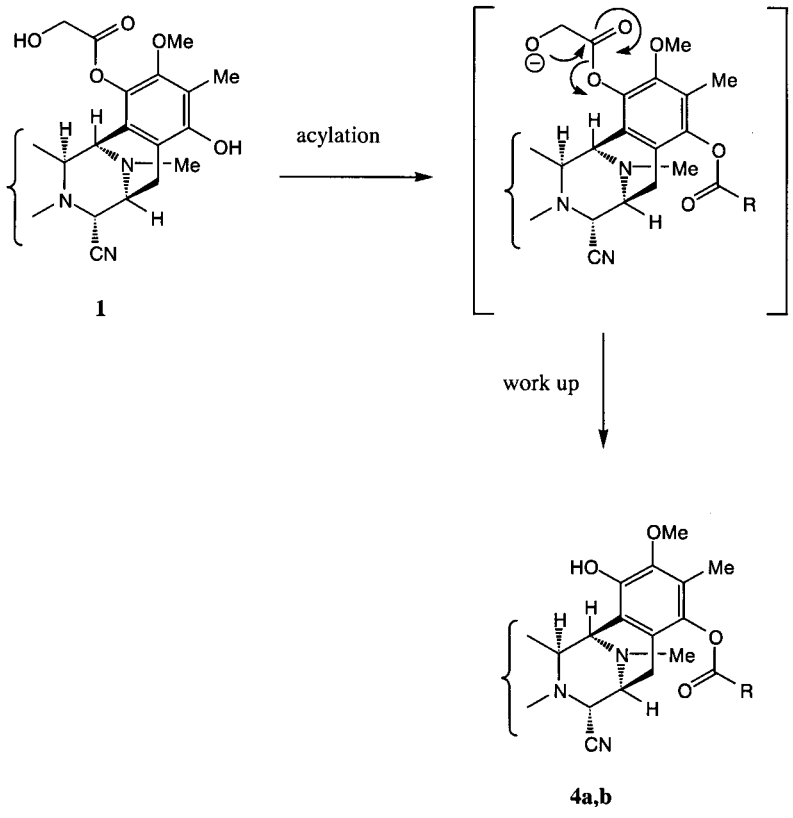
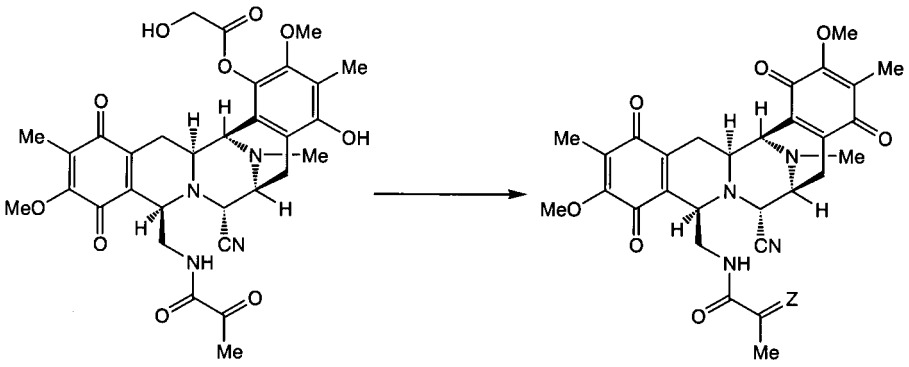



**Chart 2.**

Numerous efforts to convert **1** to the corresponding methyl derivative were unsuccessful; only polar polymeric materials were obtained. Treatment of **1** with a large excess of acetic anhydride in dry pyridine, however, gave the diacetate (**3a**) in 68% yield (Chart 1). On the other hand, acetylation of **1** with acetic anhydride (1.8 equiv.) in the presence of 4-dimethylaminopyridine (DMAP) in dry pyridine afforded the monoacetate (**4a**) in 54.9% yield along with **3a** (38.0%). We had hoped that the bulky reagent would exert enough steric influence on the course of acylation at the C-4 position. Indeed, reaction of **1** with pivaloyl chloride (2.2 equiv.) and base in $CH_2Cl_2$ afforded **4b** (46.1%) and **3b** (9.0%). There were no crystals of the four products, however characterization of **4a** and **4b** by extensive NMR measurements (including COSY, HMQC, and HMBC techniques) established unexpected results.[‡] Analysis of the $^1H$ and $^{13}C$ NMR and high-resolution MS data of **4a** and **4b** suggested the formulas of $C_{31}H_{34}N_4O_9$ and $C_{34}H_{40}N_4O_9$ for compounds **4a** and **4b**, respectively. The $^1H$- and $^{13}C$ NMR spectral data of **4a** and **4b** were very similar, with the major difference being the presence of signals attributable to an acetyl group in **4a** [$^1H$: $\delta$ 2.32 (3H, s); $^{13}C$: $\delta$ 20.5 (q) and 169.8 (s)] and to a pivaloyl group in **4b** [$^1H$: $\delta$ 1.38 (9H, s); $^{13}C$: $\delta$ 27.3 (q), 39.4 (s), and 176.8 (s)]. Both compounds have a sharp phenolic OH signal (**4a**: $\delta$ 5.73, **4b**: $\delta$ 5.69) with an NOE enhancement of

[‡] Acylation of **1** with 4-bromobenzyl chloride and base gave the diacylated compound (**3**: R=*p*-Br–$C_6H_4CH_2$: 89%), however we have not been able to obtain any crystals for X-ray analysis.



**saframycin R (1)**

**2: Z = O (saframycin A)**
**6: Z = $(OMe)_2$**

**Chart 3.**

the 2-$OCH_3$ methyl protons (**4a**: $\delta$ 3.81, **4b**: $\delta$ 3.81). Thus, the OH group must be orientated at C-1 in both **4a** and **4b**. This is consistent with the long-range $^{1}H-^{13}C$ correlations between C-1 (**4a**: $\delta$ 144.7, **4b**: $\delta$ 144.5) and H-15 (**4a**: $\delta$ 4.18, **4b**: $\delta$ 4.19). Selected HMBC correlations data in compound **4a** were shown in Fig. 2 and a probable mechanistic pathway for the formation of the acetates **4a** and **4b** is shown in Chart 2. The acylation was especially slow for the sterically crowded alcohol, and the initial attack of alkoxide to the carbonyl in glycolic ester afforded **4a** and **4b**. The possibility of preparing **3b** and **4b** from **3a** and **4a** by regioselective hydrolysis was excluded because the diacylated compounds **3a** and **4a** were very stable in organic base (such as triethylamine, DMAP).[§] Treatment of **4a** with acetic anhydride in pyridine afforded the diacetate (**5**) in 83.6% yield.

Finally, we examined the transformation of saframycin R (**1**) to saframycin A (**2**) (Chart 3). Several common methods of effecting hydrolysis were eliminated due to their ineffectiveness. Numerous attempts at this conversion under aqueous acidic or basic conditions were totally unsuccessful because of the labile nature of the quinone. Treatment of **1** with concentrated $H_2SO_4$ in methanol at 60°C for 24 h afforded saframycin A (**2**) in 19.9% yield, and the major product was the ketal (**6**) in 71.7% yield. The structure proposed for **6** was supported by the $^{13}C$ NMR spectrum, which showed a peak at $\delta$ 100.3 assigned to the ketal carbon. In addition, the $^{1}H$ NMR spectrum showed four methoxyl methyl signals at $\delta$ 2.88, 3.02, 4.06, and 4.08.[9] Accordingly, this problem was solved as follows: The reaction of **1** with a high excess triethylamine and DMAP in $CH_2Cl_2$ at room temperature for 40 h gave **2** in 44.7% yield. The synthetic **2** was identical in all respects with an authentic sample.

Saframycin R (**1**) is the first example of a latent hydroquinone at the A ring. The biological properties of **4a** and **4b** will be reported elsewhere.

[§] During the reaction, we detected a highly polar initial product along with a less polar the diacylated compound (**3a** and **4a**) using TLC. After work up, however, the highly polar compound was transformed to the final product (**3b** and **4b**).

## Experimental

IR spectra were measured in $CHCl_3$ with a Hitachi 260 spectrophotometer. $^{1}H$ spectra were measured at 270 MHz with a JEOL JNM-EX 270 spectrometer. $^{13}C$ NMR were recorded at 67.5 MHz (JEOL JNM-EX 270) and 125 MHz (JEOL JNM-LA 500) (multiplicity determined from off-resonance decoupling or distortionless enhancement by polarization transfer (DEPT) spectra). NMR spectra were measured in $CDCl_3$, and chemical shifts were recorded in $\delta_H$ values relative to internal $(CH_3)_4Si$ as a standard. Mass spectra were recorded on a JMS-DX 302 mass spectrometer. Optical rotation $[\alpha]_D$ measurements were made on a Horiba-SEPA-200 automatic digital polarimeter at 23°C. All reactions were conducted under an argon atmosphere. Dry solvents and reagents were obtained using standard procedures. Anhydrous sodium sulfate was used for drying organic solvent extracts. Removal of the solvent was done with a rotary evaporator and, finally, under high vacuum. Column chromatography was performed with E. Merck Silica gel 60 (70–230 mesh).

**Preparation of saframycin R (1).** An stocked saframycin R (**1**) was slightly impure and contained a few degradation products. It was purified by preparative thin layer chromatography on silica gel plates (E. Merck, No. 5715: solvent 1:2 benzene–ethyl acetate) immediately before use. The pure **1** as a pale yellow amorphous powder, showed $[\alpha]_D=-79.2°$ (*c* 0.6, $CHCl_3$) and its IR, MS data were identical with reported values. $^{1}H$- and $^{13}C$ NMR data were shown in Table 1. IR ($CHCl_3$) 3600w, 3400, 1770, 1725w, 1685, 1660, 1620 $cm^{-1}$; MS *m/z* (relative intensity) 622 ($M^+$, 5), 524 (10), 522 ($M^+-100$, 12), 318 (44), 279 (53), 278 (100), 220 (64), 205 (17), 204 (16), 78 (98), 77 (17), 57 (12), 44 (58); high-resolution EIMS calcd for $C_{31}H_{34}N_4O_{10}$ 622.2275, found 622.2280; Positive ion FAB-MS (magic Bullet) *m/z* 623 ($M^+$ + H), 596, 278, 220.

**Acetylation of 1.** *Method A:* Acetic anhydride (0.4 mL) was added to a solution of (−)-**1** (31.1 mg, 0.5 mmol) in dry pyridine (1.0 mL), and the mixture was stand at room temperature for 1 h. The reaction mixture was concentrated in vacuo. The residue was diluted with water (10 mL), and the mixture was extracted with chloroform (10 mL×3). The

combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give the residue (38.1 mg). Chromatography on a silica gel (9 g) column with 2:1 benzene–ethyl acetate afforded the diacetate **3a** (24.0 mg, 68.0%) as pale yellow amorphous powder: $[\alpha]_D=-77.1°$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 3370, 1745, 1675, 1655 $cm^{-1}$; $^1H$ NMR $\delta$ ($CDCl_3$) 1.42 (1H, ddd, *J*=17.2, 11.2, 2.6 Hz, 14-Hβ), 1.91 (3H, s, 12-$CH_3$), 2.10 (3H, s, $COCOCH_3$), 2.10 (1H, d, *J*=18.1 Hz, 5-Hβ), 2.18 (3H, s, 3-$CH_3$), 2.29 (3H, s, $NCH_3$), 2.32, 2.35 (each 3H, s, $COCH_3$), 2.88 (1H, dd, *J*=17.2, 3.0 Hz, 14-Hα), 2.94 (1H, dd, *J*=18.1, 7.3 Hz, 5-Hα), 2.94 (1H, signals overlap with 5-Hα, 17-H), 3.16 (1H, ddd, *J*=11.2, 3.0, 2.6 Hz, 14a-H), 3.41 (1H, d like, 6-H), 3.72 (1H, ddd, *J*=13.9, 9.2, 1.7 Hz, 17-H), 3.77 (3H, s, 2-$OCH_3$), 3.79 (1H, dd, *J*=2.6, 0.5 Hz, 15-H), 3.92 (2H, s like, 7-H and 9-H), 4.07 (3H, s, 11-$OCH_3$), 4.87, 4.96 (each 1H, d, *J*=16.2 Hz, OCOCH), 6.88 (1H, br s, NH); $^{13}C$ NMR $\delta$ ($CDCl_3$) 8.6 (q, 12-$CH_3$), 9.8 (q, 3-$CH_3$), 20.4, 20.6 (each q, OCO*C*$H_3$), 21.4 (t, C-5), 23.8 (t, C-14), 24.4 (q, COCO*C*$H_3$), 41.5 (q, $NCH_3$), 42.0 (t, 17-C), 54.5 (d, C-6), 54.5 (d, C-14a), 56.6 (d, C-9), 56.6 (d, C-15), 59.5 (d, C-7), 60.8 (t, OCO*C*$H_2$), 61.0 (q, 11-$OCH_3$), 61.1 (q, 2-$OCH_3$), 117.0 (s, CN), 122.9 (s), 123.1 (s), 123.6 (s), 125.3 (s), 127.3 (s, C-12), 136.7 (s, C-9a), 139.7 (s, C-13a), 144.7 (s), 148.7 (s), 156.4 (s, C-11), 161.3 (s, *C*OCOCH$_3$), 166.2 (s, O*C*OCH$_2$), 169.5, 170.7 (each s, O*C*OCH$_3$), 180.5 (s, C-10), 185.8 (s, C-13), 195.6 (s, CO*C*OCH$_3$); MS *m/z* (relative intensity) 706 ($M^+$, 2), 608 (20), 607 (28), 606 ($M^+-100$, 38), 581 (20), 403 (11), 402 (46), 363 (32), 362 (100), 348 (16), 320 (11), 262 (28), 229 (22), 218 (13), 204 (14), 43 (18); high-resolution EIMS calcd for $C_{35}H_{38}N_4O_{12}$ 706.2486, found 706.2484.

*Method B:* Acetic anhydride (4.7 μL, 0.050 mmol) was added to a stirred solution of (−)-**1** (17.4 mg, 0.028 mmol), triethylamine (78.0 μL, 0.560 mmol), and DMAP (6.8 mg, 0.056 mmol) in dry dichloromethane (14 mL), and the stirring was continued at room temperature for 48 h. The reaction mixture was diluted with 5% $NaHCO_3$ (10 mL), and then extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give the residue (22.7 mg). This residue was subjected to chromatography on preparative layer silica gel plates (solvent 1:2 hexane–ethyl acetate) to afford **3a** (7.5 mg, 38.0%) and **4a** (9.3 mg, 54.9%) as pale yellow amorphous powder: *N*-[4-acetyl-7-cyano-6,7,9,10,13,14,14a,15-octahydro-1-hydroxy-2,11-dimethoxy-3,12,16-trimethyl-10,13-dioxo-6α,7α,9α,14aα,15α-6,15-imino-5*H*-isoquino[3,2-*b*][3]benzazocin-9-yl]-methyl]-2-oxopropanamide (**4a**): $[\alpha]_D=-74.5°$ (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 3570, 3410, 1760, 1710, 1690, 1670 $cm^{-1}$; $^1H$ NMR $\delta$ ($CDCl_3$) 1.48 (1H, ddd, *J*=17.5, 11.2, 2.3 Hz, 14-Hβ), 1.90 (3H, s, 12-$CH_3$), 2.10 (3H, s, $COCOCH_3$), 2.13 (1H, d, *J*=18.5 Hz, 5-Hβ), 2.17 (3H, s, 3-$CH_3$), 2.32 (3H, s, $COCH_3$), 2.37 (3H, s, $NCH_3$), 2.91 (1H, dd, *J*=18.5, 6.9 Hz, 5-Hα), 2.91 (1H, signals overlap with 5-Hα, 17-H), 3.01 (1H, dd, *J*=17.5, 2.6 Hz, 14-Hα), 3.16 (1H, ddd, *J*=11.2, 2.6, 2.6 Hz, 14a-H), 3.39 (1H, d like, 6-H), 3.73 (1H, ddd, *J*=13.5, 8.9, 1.7 Hz, 17-H), 3.82 (3H, s, 2-$OCH_3$), 3.93 (2H, s like, 7-H and 9-H), 4.06 (3H, s, 11-$OCH_3$), 4.18 (1H, dd, *J*=2.6, 0.5 Hz, 15-H), 5.73 (1H, s, OH), 6.88 (1H, br s, NH); $^{13}C$ NMR $\delta$ ($CDCl_3$) 8.6 (q, 12-$CH_3$), 9.8 (q, 3-$CH_3$), 20.5 (q, $OCOCH_3$), 21.4 (t, C-5), 23.9 (t, C-14), 24.4 (q, COCO*C*$H_3$), 41.7 (q, $NCH_3$), 41.9 (t, C-17), 54.6 (d, C-6), 54.9 (d, C-14a), 55.7 (d, C-15), 56.5 (d, C-9), 59.4 (d, C-7), 61.0 (q, 11-$OCH_3$), 61.1 (q, 2-$OCH_3$), 115.6 (s, C-15a), 117.2 (s, CN), 122.7 (s, C-3), 122.7 (s, C-4a), 128.5 (s, C-12), 136.0 (s, C-9a), 139.1 (s, C-4), 140.6 (s, C-13a), 143.5 (s, C-2), 144.7 (s, C-1), 156.3 (s, C-11), 161.6 (s, *C*OCOCH$_3$), 169.8 (s, O*C*OCH$_3$), 180.6 (s, C-10), 186.0 (s, C-13), 195.5 (s, CO*C*OCH$_3$); MS *m/z* (relative intensity) 606 ($M^+$, 2), 509 (11), 508 (34), 507 (10), 506 ($M^+-100$, 25), 483 (22), 481 (19), 302 (27), 264 (11), 263 (27), 262 (100), 248 (12), 245 (14), 229 (14), 220 (30), 206 (12), 205 (17), 204 (20), 171 (15), 171 (15), 149 (25), 107 (28), 97 (12), 91 (37), 83 (10), 71 (17), 69 (20), 59 (16), 57 (29), 56 (10), 55 (20), 43 (15), 41 (18); high-resolution EIMS calcd for $C_{31}H_{34}N_4O_9$ 606.2326, found 606.2330.

**Acylation of 1 with pivaloyl chloride.** Trimethylacetyl chloride (pivaloyl chloride, 15.4 μL, 0.125 mmol) was added to a solution of (−)-**1** (16.7 mg, 0.027 mmol), triethylamine (34.8 μL, 0.250 mmol), and DMAP (1.2 mg, 0.010 mmol) in dry dichloromethane (5.0 mL), and the mixture was stand at room temperature for 24 h. The reaction mixture was diluted with 5% $NaHCO_3$ (10 mL), and then extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give the residue (24.9 mg). Chromatography on a silica gel (9 g) column with 3:1 hexane–ethyl acetate afforded **3b** (15.6 mg, 73.7%) as pale yellow amorphous powder. Further elution with 2:1 hexane–ethyl acetate afforded **4b** (4.0 mg, 23.0%) as pale yellow amorphous powder.

**Compound 3b.** $[\alpha]_D=-56.9°$ (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) 3550, 3390, 1740, 1680, 1665 $cm^{-1}$; $^1H$ NMR $\delta$ ($CDCl_3$) 1.27 and 1.38 (each 9H, s, $C(CH_3)_3$), 1.45 (1H, ddd, *J*=17.5, 10.0, 2.0 Hz, 14-Hβ), 1.91 (3H, s, 12-$CH_3$), 2.01 (1H, d, *J*=18.1 Hz, 5-Hβ), 2.09 (3H, s, $COCOCH_3$), 2.16 (3H, s, 3-$CH_3$), 2.31 (3H, s, $NCH_3$), 2.89–2.96 (2H, m, signals overlap with 5-Hα, and 17-H), 3.01 (1H, dd, *J*=17.5, 2.6 Hz, 14-Hα), 3.18 (1H, ddd, *J*=11.2, 2.6, 2.6 Hz, 14a-H), 3.40 (1H, d like, 6-H), 3.72 (1H, ddd, *J*=13.9, 9.2, 1.7 Hz, 9-CH), 3.77 (3H, s, 2-$OCH_3$), 3.86 (1H, dd, *J*=2.6, 0.5 Hz, 15-H), 3.92 (2H, s like, 7-H and 17-H), 4.07 (3H, s, 11-$OCH_3$), 6.91 (1H, br s, NH); $^{13}C$ NMR $\delta$ ($CDCl_3$) 8.6 (q, 12-$CH_3$), 9.7 (q, 3-$CH_3$), 21.4 (t, C-5), 22.8 (t, C-14), 24.4 (q, COCO*C*$H_3$), 27.0 and 27.3 (each s, $C(CH_3)_3$), 38.7, 39.5 (each s, $C(CH_3)_3$), 41.4 (q, $NCH_3$), 42.2 (t, C-17), 54.5 (d, C-6), 54.5 (d, C-14a), 56.6 (d, C-9), 56.6 (d, C-15), 59.5 (d, C-7), 60.6 (t, OCO*C*$H_2$), 61.0 (q, 11-$OCH_3$), 61.1 (q, 2-$OCH_3$), 117.0(s, CN), 122.8 (s), 122.9 (s), 123.0 (s), 125.3 (s), 127.3 (s, C-12), 136.3 (s, C-9a), 139.9 (C-13a), 144.6 (s), 148.8 (s), 156.4 (s, C-11), 161.5 (s, *C*OCOCH$_3$), 166.2 (s, OCOCH$_2$), 176.6, 178.0 (each s, OCOC(CH$_3$)$_3$), 180.5 (s, C-10), 185.8 (s, C-13), 195.4 (s, CO*C*OCH$_3$); MS *m/z* (relative intensity) 790 ($M^+$, 2), 693 (45), 692 (45), 691 (35), 690 ($M^+-100$, 35), 674 (13), 667 (22), 666 (11), 665 (26), 487 (12), 486 (38), 448 (12), 447 (36), 446 (100), 362 (15), 361 (11), 344 (17), 305 (15), 304 (59), 220 (26), 219 (12), 218 (34), 205 (11), 204 (18), 149 (13), 97 (10), 85 (10), 83 (10), 71 (14), 69 (15), 59 (11); high-resolution EIMS calcd for $C_{41}H_{50}N_4O_{12}$ 790.3425, found 790.3427.

**Compound 4b.** $[\alpha]_D$=−55.3° (*c* 0.35, $CHCl_3$); IR ($CHCl_3$) 3570, 3410, 1760, 1710, 1690, 1670 $cm^{-1}$; $^1H$ NMR δ ($CDCl_3$) 1.38 (9H, s, $C(CH_3)_3$), 1.48 (1H, ddd, *J*=17.5, 11.2, 2.3 Hz, 14-Hβ), 1.91 (3H, s, 12-$CH_3$), 2.08 (3H, s, $COCOCH_3$), 2.13 (1H, d, *J*=18.5 Hz, 5-Hβ), 2.14 (3H, s, 3-$CH_3$), 2.37 (3H, s, $NCH_3$), 2.91 (1H, dd, *J*=18.5, 6.9 Hz, 5-Hα), 2.91 (1H, signals overlap with 5-Hα, 17-H), 3.01 (1H, dd, *J*=17.5, 2.6 Hz, 14-Hα), 3.18 (1H, ddd, *J*=11.2, 2.6, 2.6 Hz, 14a-H), 3.40 (1H, d like, 6-H), 3.73 (1H, ddd, *J*=13.5, 8.9, 1.7 Hz, 17-H), 3.81 (3H, s, 2-$OCH_3$), 3.93 (2H, s like, 7-H and 9-H), 4.06 (3H, s, 11-$OCH_3$), 4.19 (1H, dd, *J*=2.6, 0.5 Hz, 15-H), 5.69 (1H, s, OH), 6.91 (1H, br s, NH); $^{13}C$ NMR δ ($CDCl_3$) 8.6 (q, 12-$CH_3$), 9.8 (q, 3-$CH_3$), 21.5 (t, C-5), 23.9 (t, C-14), 24.5 (q, $COCOCH_3$), 27.3 (q, $C(CH_3)_3$), 39.4 (s, $C(CH_3)_3$), 41.7 (q, $NCH_3$), 42.2 (t, C-17), 54.7 (d, C-6), 54.9 (d, C-14a), 55.9 (d, C-15), 56.5 (d, C-9), 59.6 (d, C-7), 61.0 (q, 11-$OCH_3$), 61.1 (q, 2-$OCH_3$), 115.6 (s, C-15a), 117.2 (s, CN), 122.7 (s, C-3), 122.8 (s, C-4a), 127.3 (s, C-12), 136.2 (s, C-9a), 139.1 (s, C-4), 140.6 (s, C-13a), 143.5 (s, C-2), 144.5 (s, C-1), 156.4 (s, C-11), 161.6 (s, $COCOCH_3$), 176.8 (s, $OCO(CH_3)_3$), 180.6 (s, C-10), 186.0 (s, C-13), 195.4 (s, $COCOCH_3$); MS *m*/*z* (relative intensity) 648 ($M^+$, 6), 550 (17), 549 (30), 548 ($M^+$−100, 77), 524 (11), 523 (30), 345 (15), 344 (64), 305 (28), 304 (100), 290 (17), 259 (15), 243 (10), 220 (30), 218 (14), 205 (17), 204 (23), 149 (12), 69 (12), 57 (41), 55 (11), 43 (11); high-resolution EIMS calcd for $C_{34}H_{40}N_4O_9$ 648.2795, found 648.2800.

The same procedure as described above, but using (−)-**1** (16.7 mg, 0.0268 mmol) with pivaloyl chloride (7.4 μL, 0.06 mmol), triethylamine (69.6 μL, 0.50 mmol), and DMAP (6.1 mg, 0.05 mmol) in dry dichloromethane (14 mL) at room temperature for 24 h gave **3b** (1.9 mg, 9.0%) and **4b** (8.0 mg, 46.1%).

**Acetylation of 4a.** Acetic anhydride (0.2 mL) was added to a solution of **4a** (8.5 mg, 0.014 mmol) in dry pyridine (0.5 mL), and the mixture was stand at room temperature for 14 h. The reaction mixture was concentrated in vacuo. The residue was diluted with water (10 mL), and the mixture was extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give the residue (10.3 mg). This residue was subjected to chromatography on preparative layer silica gel plates (solvent 1:1 hexane–ethyl acetate) to afford **5** (7.6 mg, 83.6%) as pale yellow amorphous powder: *N*-[1,4-diacetyl-7-cyano-6,7,9,10,13,14,14a,15-octahydro -2,11-dimethoxy-3,12,16-trimethyl-10,13-dioxo-6α,7α,9α,14aα,15α-6,15-imino-5*H*-isoquino[3,2-*b*][3]benzazocin-9-yl]-methyl]-2-oxopropanamide (**5**): IR ($CHCl_3$) 3400, 1750, 1690, 1670 $cm^{-1}$; $^1H$ NMR δ ($CDCl_3$) 1.46 (1H, ddd, *J*=17.5, 11.2, 2.3 Hz, 14-Hβ), 1.91 (3H, s, 12-$CH_3$), 2.10 (3H, s, $COCOCH_3$), 2.13 (1H, d, *J*=18.5 Hz, 5-Hβ), 2.18 (3H, s, 3-$CH_3$), 2.34, 2.35 (each 3H, s, $COCH_3$), 2.42 (3H, s, $NCH_3$), 2.87–3.01 (3H, m, 5-Hα, 17-H, and 14-Hα), 3.16 (1H, ddd, *J*=11.2, 3.0, 2.6 Hz, 14a-H), 3.43 (1H, d like, 6-H), 3.71 (1H, ddd, *J*=13.2, 9.8, 2.0 Hz, 17-H), 3.73 (1H, dd, *J*=2.0, 0.5 Hz, 15-H), 3.78 (3H, s, 2-$OCH_3$), 3.93 (2H, s like, 7-H and 9-H), 4.07 (3H, s, 11-$OCH_3$), 6.87 (1H, br s, NH); $^{13}C$ NMR δ ($CDCl_3$: all unsaturated carbon peaks could not determined because of the limited amount of sample available) 8.6 (q, 12-$CH_3$), 9.8 (q, 3-$CH_3$), 20.6, 20.7 (each q, $OCOCH_3$), 21.5 (t, C-5), 23.8 (t, C-14), 24.5 (q, $COCOCH_3$), 40.9 (q, $NCH_3$), 41.5 (t, 9-$CH_2$), 54.5 (d, C-6), 57.0 (d, C-9), 57.2 (d, C-15), 57.5 (d, C-14a), 59.4 (d, C-7), 60.9 (q, 11-$OCH_3$), 61.0 (q, 2-$OCH_3$), MS *m*/*z* (relative intensity) 648 ($M^+$, 3), 551 (13), 550 (40), 549 (30), 548 ($M^+$−100, 33), 525 (11), 523 (21), 344 (44), 305 (32), 304 (100). 302 (11), 290 (19), 262 (35), 246 (16), 229 (10), 220 (26), 205 (11), 204 (13), 43 (12); high-resolution EIMS calcd for $C_{33}H_{36}N_4O_{10}$ 648.2432, found 648.2424.

**Transformation of saframycin R (1) to saframycin A (2).** *Method A:* A solution of (−)-**1** (9.9 mg, 0.0159 mmol) was stirred with triethylamine (44.4 μL, 0.319 mmol) and DMAP (3.9 mg, 0.0319 mmol) in dry dichloromethane (8 mL) at room temperature for 40 h. The reaction mixture was diluted with 1N HCl (10 mL), and extracted with chloroform (10 mL×3). The combined extracts were washed with water (10 mL), dried, and concentrated in vacuo to give the neutral fraction (3.2 mg). This fraction was subjected to chromatography on preparative layer silica gel (Merck 5715: solvent 1:2 hexane–ethyl acetate) to give the starting material (2.6 mg, 26.3% recovery). The acidic aqueous layer was made alkaline with saturated $NaHCO_3$ solution and extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo. The residue (6.1 mg) was subjected to chromatography on preparative layer silica gel (Merck 5715: solvent 1:1 hexane–ethyl acetate) to give saframycin A (**2**: 4.0 mg, 44.7%) as dark yellow amorphous powder, which was identical in all respects with an authentic sample.

*Method B:* Concentrated $H_2SO_4$ (0.1 mL) was added to a stirred solution of (−)-**1** (16.7 mg, 0.0268 mmol) in methanol (5.0 mL), and the reaction mixture was heated at 70°C for 5 h. The reaction mixture was diluted with 5% $NaHCO_3$ (15 mL), and extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dired, and concentrated in vacuo to give the residue (22.4 mg). Chromatography on a silica gel (8 g) column with 2:1 hexane–ethyl acetate afforded saframycin A (3.0 mg, 19.9%). Further elution with 1:1 hexane–ethyl acetate afforded **6** (11.7 mg, 71.7%) as pale yellow amorphous powder.

**(−)-Saframycin A dimethylketal (6).** $[\alpha]_D$=−24.9° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3450, 1690, 1660, 1620 $cm^{-1}$; $^1H$ NMR δ ($CDCl_3$) 1.18 (3H, s, C–$CH_3$), 1.31 (1H, ddd, *J*=17.5, 11.2, 2.6 Hz, 14-Hβ), 1.87, 1.98 (each 3H, s, 3- and 12-$CH_3$), 2.23 (1H, d, *J*=21.1 Hz, 5-Hβ), 2.32 (3H, s, $NCH_3$), 2.86 (1H, dd, *J*=21.1, 8.9 Hz, 5-Hα), 2.88 (3H, s, $OCH_3$), 2.89 (1H, dd, *J*=17.5, 2.6 Hz, 14-Hα), 2.92 (1H, ddd, *J*=13.0, 4.0, 3.3 Hz, 17-H), 3.02 (3H, s, $OCH_3$), 3.12 (1H, ddd, *J*=11.2, 3.0, 2.6 Hz, 14a-H), 3.46 (1H, ddd, *J*=8.9, 2.3, 0.5 Hz, 6-H), 3.95 (1H, ddd, *J*=13.0, 10.2, 1.7 Hz, 17-H), 3.96 (1H, s like, 9-H), 4.04 (1H, d, *J*=2.3 Hz, 7-H), 4.05 (1H, dd, *J*=3.0, 0.5 Hz, 15-H), 4.06, 4.08 (each 3H, s, 2- and 11-$OCH_3$), 6.57 (1H, dd, *J*=10.2, 3.3 Hz, NH); $^{13}C$ NMR δ ($CDCl_3$) 8.5 (q, quinone-$CH_3$), 8.8 (q, quinone-$CH_3$), 21.1 (q, C–$CH_3$), 21.6 (t, C-5), 25.3 (t, C-14), 40.8 (t, C-17), 41.6 (q, $NCH_3$), 49.3 (q, ketal-$OCH_3$), 49.6 (q, ketal-$OCH_3$), 53.9 (d, C-14a), 54.2 (d, C-9), 54.4 (d,

C-6), 57.3 (d, C-15), 58.3 (d, C-7), 61.1 (q, quinone-$OCH_3$), 61.1 (q, quinone-$OCH_3$), 100.3 (s, ketal-C), 116.6 (s, CN), 127.0 (s), 128.1 (s), 135.8 (s), 136.4 (s), 139.9 (s, C-4), 141.5 (s), 155.3 (s), 156.6 (s), 170.2 (s, $COC(OCH_3)_2CH_3$), 180.6 (s), 182.3 (s), 185.6 (s), 186.3 (s); MS *m/z* (relative intensity) 608 ($M^+$, 1), 464 (21), 437 (11), 245 (18), 244 (12), 243 (12), 221 (22), 220 (63), 219 (11), 218 (16), 204 (10), 203 (11), 89 (100); high-resolution EIMS calcd for $C_{31}H_{36}N_4O_9$ 608.2482., found 608.2497.

**Synthesis of saframycin A dimethylketal (6).** Concentrated $H_2SO_4$ (0.1 mL) was added to a stirred solution of (−)-2 (28.1 mg, 0.50 mmol) in methanol (5.0 mL), and the reaction mixture was heated at 70°C for 5 h. The reaction mixture was diluted with 5% $NaHCO_3$ (15 mL), and extracted with chloroform (10 mL×3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give the residue (34.5 mg). Chromatography on a silica gel (8 g) column with 2:1 hexane–ethyl acetate afforded saframycin A (4.2 mg, 14.9% recovery). Further elution with 1:1 hexane–ethyl acetate afforded **6** (17.5 mg, 57.6%) as pale yellow amorphous powder, whose spectra were identical with those of an authentic sample described above.

## Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan. We are grateful to Professor Emeritus T. Arai of Chiba University for kindly providing a sample of natural saframycin R. We acknowledge Dr K. Koyama of Meiji Pharrmaceutical University for valuable discussions. We would like thank Ms T. Kozeki and Ms A. Ohmae in the Analytical Center of this University for measurements of mass spectral data and NMR data.

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